

Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes.

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Journal of Proteome Research

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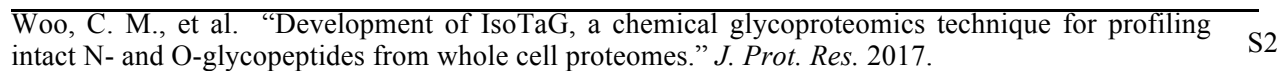
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Table S1. Full list of intact glycopeptides characterized by IsoTaG across a 15 cell line survey.

Table S2. Frequency of glycan structures observed by IsoTaG across a 15 cell line survey.

Figure S1. Fragmentation spectra of an untagged alpha-crystallin glycopeptide (AIPVS*R²⁺) by HCD and ETD. Spectra were obtained on an Orbitrap Elite. Glycosite denoted by “*”.



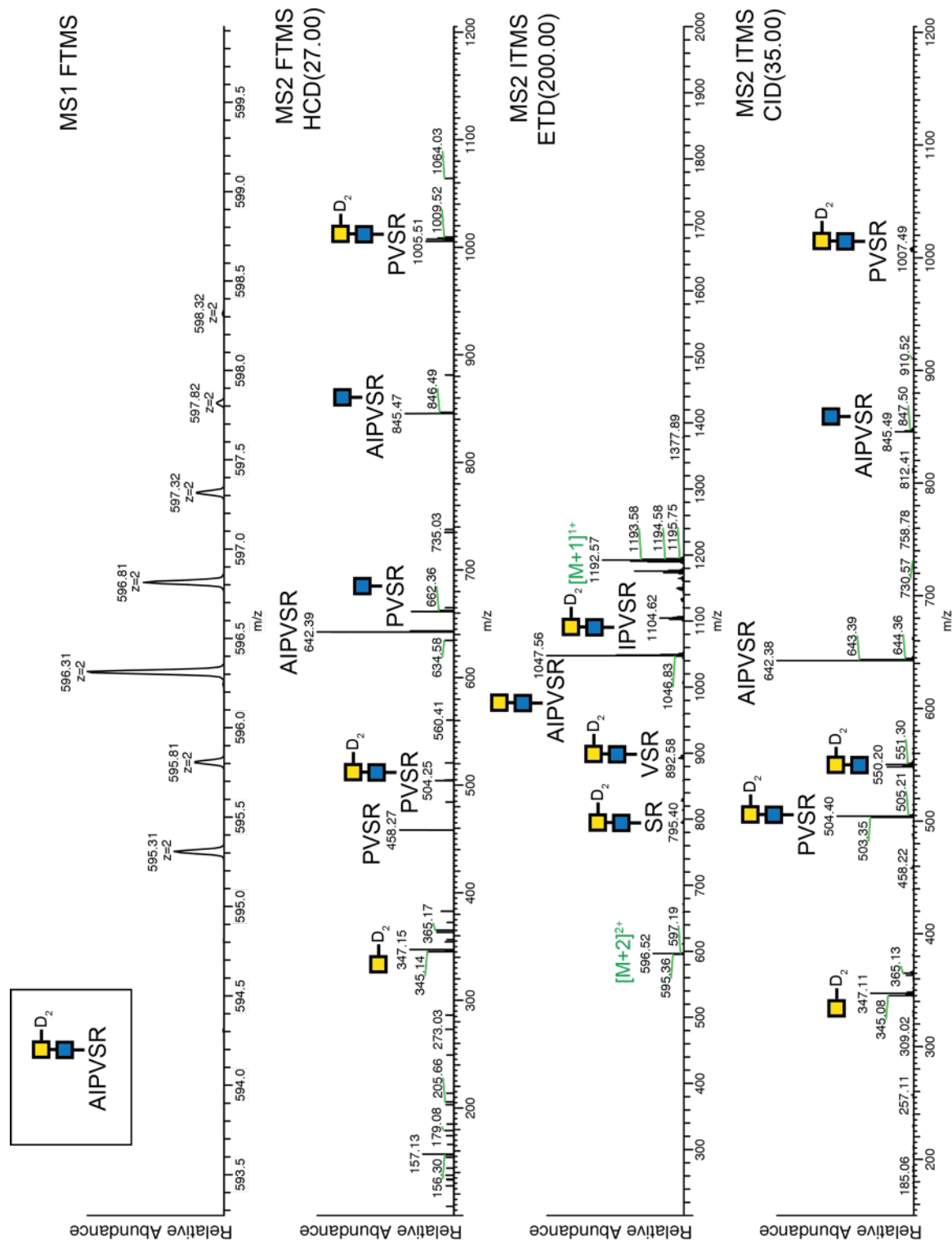


Figure S2. Fragmentation spectra of a GalNAz-labeled and probe **3** tagged alpha-crystallin glycopeptide (AIPVS*R²⁺) by HCD, ETD and CID. Spectra were obtained on an Orbitrap Elite. Glycosite denoted by “*”.

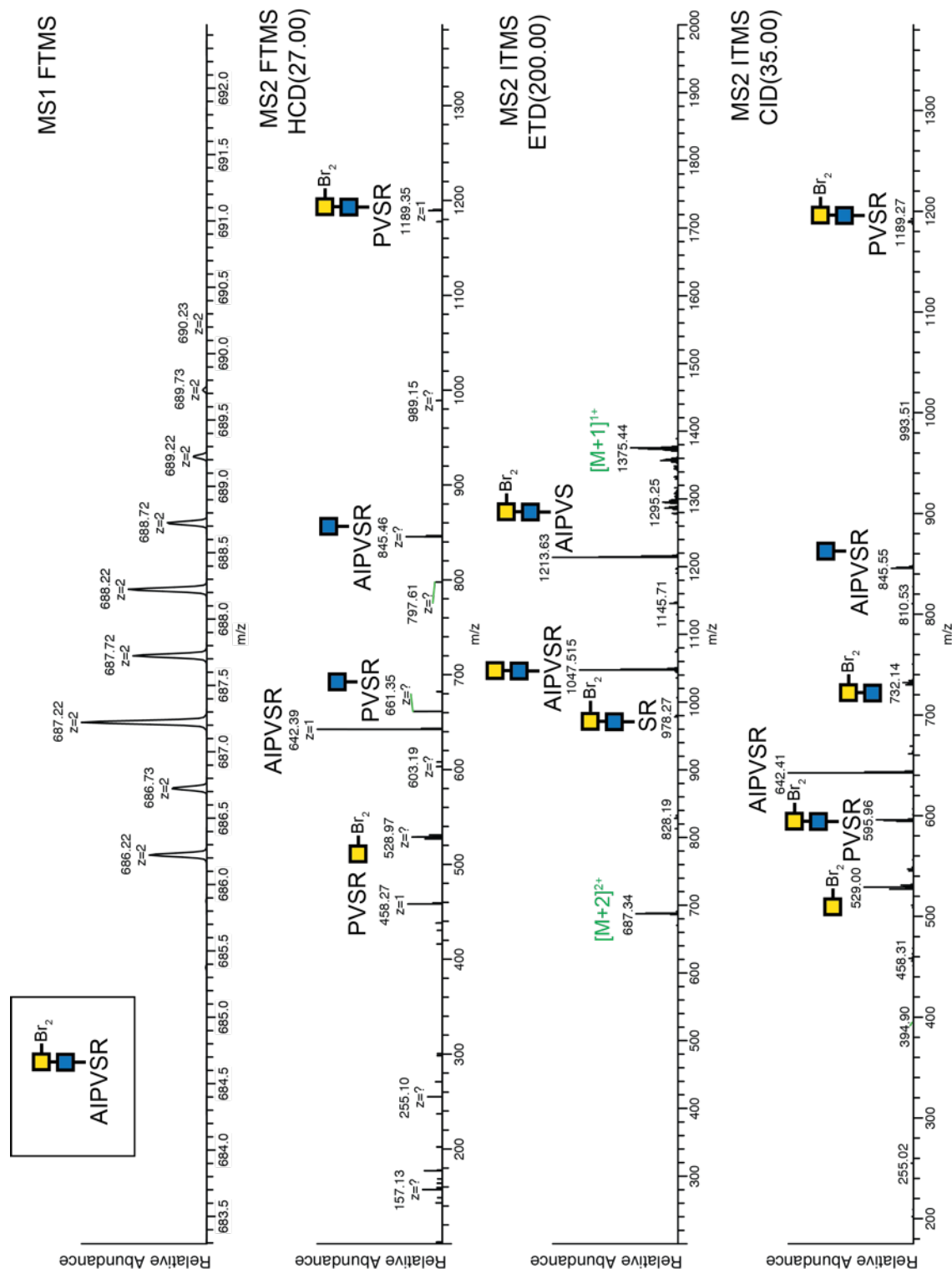


Figure S3. Fragmentation spectra of a GalNAz-labeled and dibrominated probe tagged alpha-crystallin glycopeptide (AIPVS*R²⁺) by HCD, ETD and CID. Spectra were obtained on an Orbitrap Elite. Probe was analogous to that used in (2). Glycosite denoted by “*”.

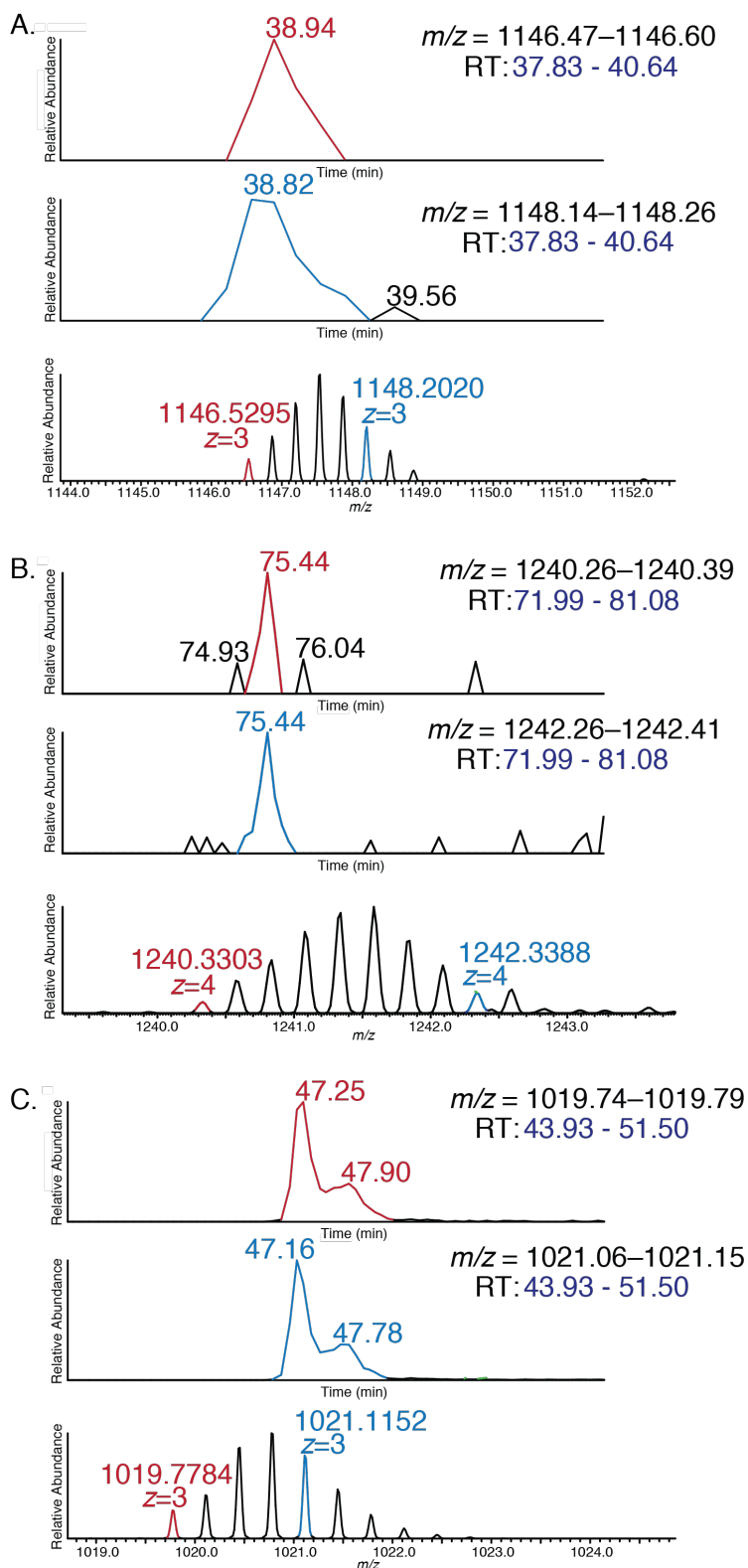


Figure S4. Nanoflow liquid chromatography retention time of two isotopes from glycopeptides tagged by probe 3. A maximum retention time shift of 0.1 m was observed. Each example has the elution time distribution color-coded to the two isotopes of the precursor in the third panel.

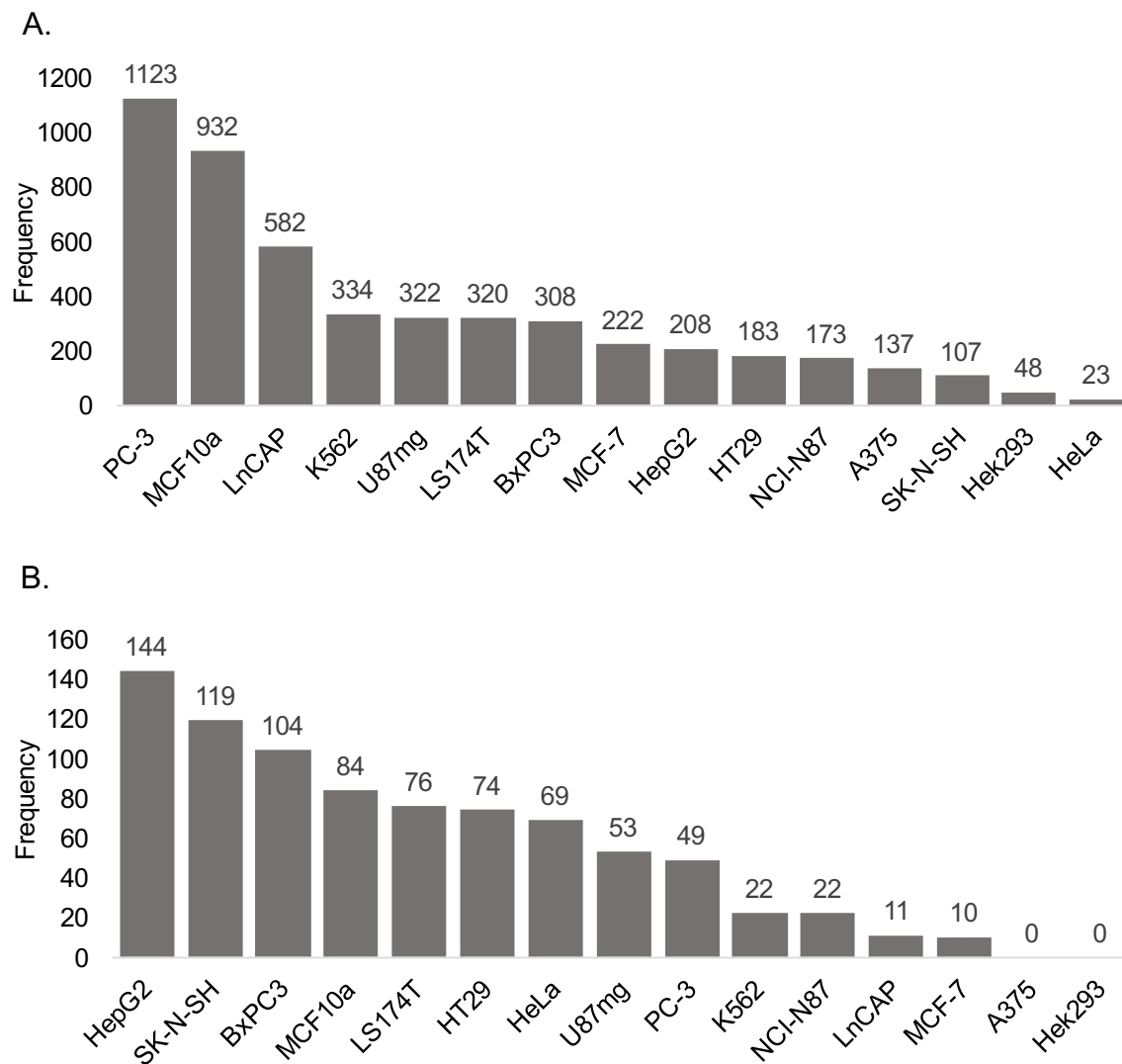


Figure S5. Spectral counts derived from isotopically recoded precursors by cell line in descending order. A. Cell lines treated with 100 μ M Ac₄GalNAz. B. Cell lines treated with 100 μ M Ac₄ManNAz.

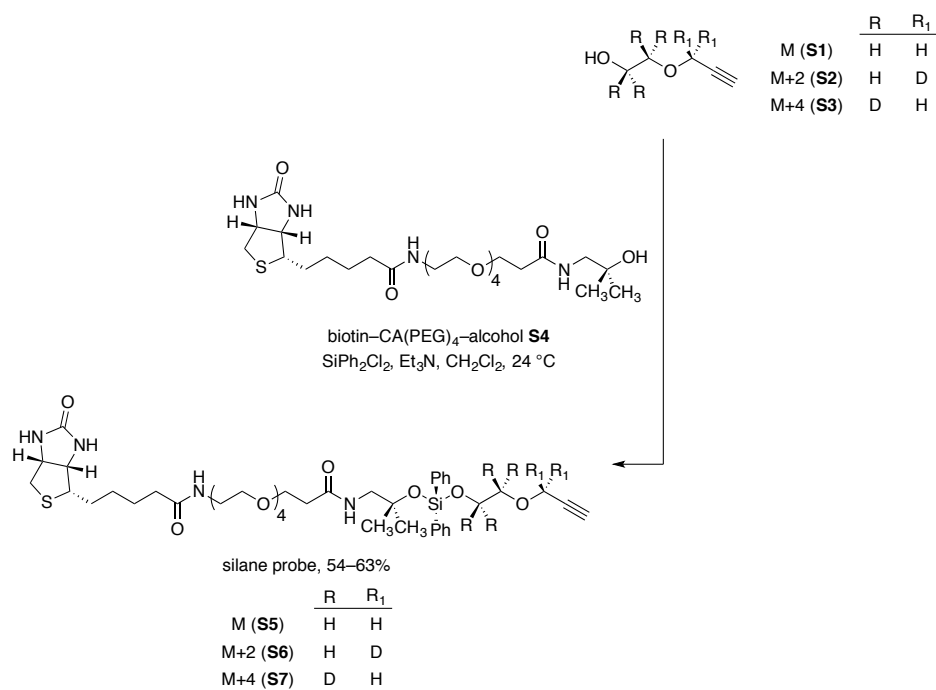


Figure S6. Synthesis of the silane probes **S5–S7**.

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation at 30–33 °C. Normal and reverse phase flash-column chromatography was performed as described by Still and co-workers (3). Normal phase purifications employ silica gel (60 Å, 40–63 µm particle size) purchased from Silicycle (Quebec, Canada). Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV) and/or submersion in aqueous ceric ammonium molybdate solution (CAM) followed by brief heating on a hot plate (120 °C, 10–15 s).

Chemical Materials. Commercial solvents and reagents were used as received with the following exceptions. Dichloromethane and *N,N*-dimethylformamide were purified according to the method of Pangborn and co-workers (4). Triethylamine was distilled from calcium hydride under an atmosphere of nitrogen immediately before use. RapiGest was prepared according to the method of Lee and co-workers (5). 3-[4-({Bis[(1-tert-butyl-1H-1,2,3-triazol-4-yl)methyl]amino}methyl)-1H-1,2,3-triazol-1-yl]propanol (BTTP) was prepared according to the method of Wu and co-workers (6). Tetraacetylated *N*-azidoacetyl galactosamine was prepared according to the method of Bertozzi and co-workers (7). Tetraacetylated *N*-azidoacetyl mannosamine was prepared according to the method of Bertozzi and co-workers (8).

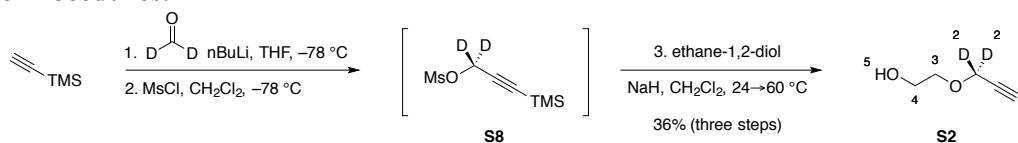
Cell Culture Materials. PC-3, MCF10a, LnCAP, K562, LS174T, NCI-N87, BxPC-3, U-87 MG, MCF-7, Hep G2, SK-N-SH, A375, HT-29, HeLa, HEK293 cell lines were obtained from the American Type Culture Collection (ATCC). LnCAP, PC-3, and BxPC-3 cells were maintained in RPMI-1640 supplemented with 10% FBS and 1% penicillin/streptomycin. K562, LS174T, NCI-N87, U-87 MG, Hep G2, SK-N-SH, A375, HT-29, HeLa, HEK293 cells were maintained in MEM supplemented with 10% FBS and 1% penicillin/streptomycin. MCF-7 cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. MCF10a cells were maintained in DMEM supplemented with 5% horse serum, 20 ng/mL human EGF, 0.5 mg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 µg/mL insulin, 1% penicillin/streptomycin. EDTA-free protease inhibitor cocktail was obtained from Roche Diagnostics (Version 11). Streptavidin–agarose beads were obtained from Thermo Scientific and washed with PBS prior to use.

Instrumentation. Proton nuclear magnetic resonance spectra (¹H NMR) were recorded at 400 or 600 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent (CHCl₃, δ 7.26; CHD₂OD, δ 3.31). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (¹³C NMR) were recorded at 100 or 125 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent (CDCl₃, δ 77.0; CD₃OD, δ 49.0). ¹³C NMR and data are represented as follows: chemical shift, carbon type [determined from HSQC]. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane. Infrared (IR) spectra were obtained using a Thermo Electron Corporation Nicolet 8500 FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm⁻¹), intensity of absorption (s = strong, m = medium, w = weak, br = broad). High-resolution mass spectrometry (HRMS) measurements were obtained at the QB3/Chemistry Mass Spectrometry Facility at the University of California, Berkeley, using a Thermo LTQ-FT mass spectrometer. Liquid chromatography-tandem mass spectrometry measurements were obtained using a Thermo Dionex UltiMate3000 RSLCnano liquid chromatograph that was connected in-line with an LTQ Orbitrap XL mass spectrometer equipped with a

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nanoelectrospray ionization (nanoESI) source (Thermo Fisher Scientific, Waltham, MA). Mass spectrometry data were analyzed with Proteome Discoverer v1.4 using SEQUEST HT and Byonic v2.0 algorithms. Mass spectrometry data are annotated as losses from the $MS^{(n-1)}$ precursor mass as follows: glycan (number of glycan units) in reverse order of the observed losses. Glycans at separate sites are separated by a comma. Glycan annotations used: Si0NeuAz = $C_{16}H_{24}N_4O_{10}$ (+432.1492), Si2NeuAz = $C_{16}H_{22}D_2N_4O_{10}$ (+434.1618), NeuAz = $C_{11}H_{16}N_4O_8$ (+332.0968), NeuAcNH₂ = $C_{11}H_{18}N_2O_8$ (+306.1063), NeuAc = $C_{11}H_{17}NO_8$ (+291.0954), Si0HexNAz = $C_{13}H_{20}N_4O_7$ (+344.1332), Si2HexNAz = $C_{13}H_{18}D_2N_4O_7$ (+346.14575), HexNAz = $C_8H_{12}N_4O_5$ (+244.0808), HexNAcNH₂ = $C_8H_{14}N_2O_5$ (+218.0903), HexNAc = $C_8H_{13}NO_5$ (+203.0794), Hex = $C_6H_{10}O_5$ (+162.0528), Fuc = $C_6H_{10}O_4$ (+146.0579).

Synthetic Procedures.



Synthesis of 2-((prop-2-yn-1-yl-1,1-d₂)oxy)ethan-1-ol (S2):

Step 1. Addition of trimethylsilyl acetylene to paraformaldehyde-d₂:

n-Butyl lithium (2.45 M, 6.00 mL, 14.7 mmol, 1.17 equiv) was added to a stirred solution of trimethylsilyl acetylene (2.10 mL, 14.7 mmol, 1.17 equiv) in tetrahydrofuran (60 mL) at -78°C . The resulting solution was stirred for 3 h at -78°C . Paraformaldehyde-d₂ (400 mg, 12.5 mmol, 1 equiv) was added to the stirred solution at -78°C . The resulting solution was warmed over 10 min to 24°C . The warmed solution was stirred for 12 h at 24°C . The product mixture was diluted sequentially with diethyl ether (100 mL) and saturated aqueous ammonium chloride solution (100 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ether (4 · 50 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated.

Step 2. Mesyl protection of the propargyl alcohol-d₂ (S8):

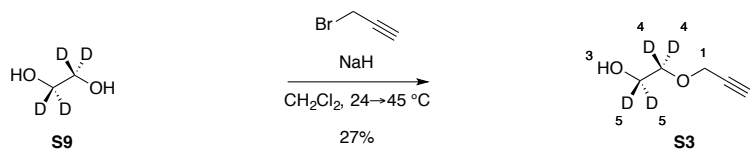
The residue obtained in the previous step was resuspended in dichloromethane (21 mL) and cooled to -78°C . Triethylamine (2.97 mL, 21.5 mmol, 2.00 equiv) and methanesulfonyl chloride (915 μL , 11.8 mmol, 1.10 equiv) were added in sequence to the stirred solution at -78°C . The resulting solution was stirred for 1 h at -78°C . The product mixture was diluted sequentially with dichloromethane (100 mL) and saturated aqueous sodium bicarbonate solution (100 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 · 50 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated.

Step 3. Synthesis of 2-((prop-2-yn-1-yl-1,1-d₂)oxy)ethan-1-ol (S2):

Sodium hydride (264 mg, 11.0 mmol, 1.10 equiv) was added to a stirred solution of ethane-1,2-diol (1.70 mL, 30.0 mmol, 3.00 equiv) in toluene (8.0 mL) at -78°C . The resulting mixture was stirred for 5 min at -78°C , and was then warmed over 10 min to 24°C . The warmed mixture was stirred for 1 h at 24°C . The mesyl alkyne **S8** (2.08 g, 15.5 mmol, 1 equiv) prepared in the previous step was added dropwise to the stirred solution. The vessel containing the mesyl alkyne **S8** was rinsed with toluene (1.0 mL) and the rinse was added to the stirred solution. The resulting solution was heated over 5 min to 60°C . The heated solution was stirred for 13 h at 60°C . The product mixture was cooled over 10 min to 24°C , and diluted sequentially with dichloromethane (50 mL) and saturated aqueous sodium bicarbonate solution (50 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 · 50 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 20% ethyl acetate–hexanes, grading to 50% ethyl acetate–hexanes, 3 steps) to afford the deuterated alkyne **S2** as a clear oil (453 mg, 36% over 3 steps).

$R_f = 0.06$ (20% ethyl acetate–hexanes; CAM). ^1H NMR (400 MHz, CDCl_3): δ 3.72 (t, 2H, $J = 4.4$ Hz, H_4), 3.61 (t, 2H, $J = 4.4$ Hz, H_3), 2.55 (br s, 1H, OH), 2.43 (s, 1H, H_1). ^2H NMR (400 MHz, CDCl_3): δ 4.19 (s, 2H, H_2). ^{13}C NMR (500 MHz, CDCl_3): δ 79.6 (C), 74.9 (CH), 71.3 (CH_2), 61.9 (CH_2). IR (ATR-FTIR), cm^{-1} : 3283 (br), 1729 (m), 1108 (s).

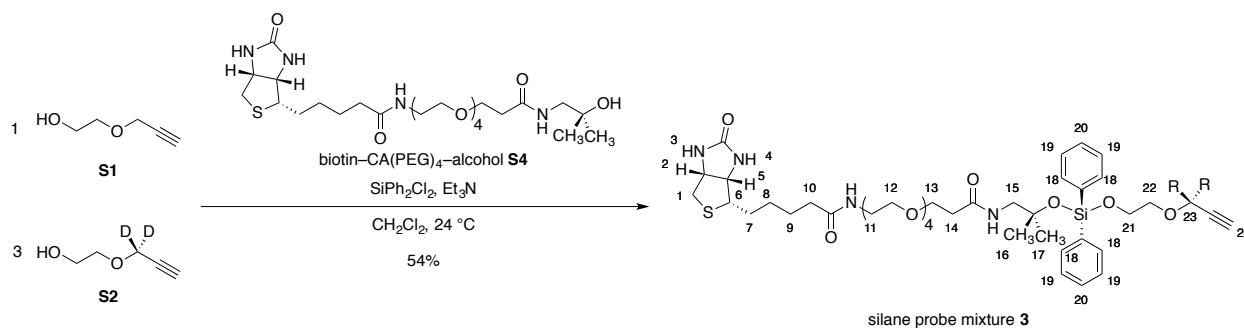
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Synthesis of (E)-2,3-Dibromo-4-(prop-2-yn-1-yloxy)but-2-en-1-ol (S3):

Sodium hydride (333 mg, 8.33 mmol, 1.10 equiv) was added to a stirred solution of ethane-*d*₄-1,2-diol (**S9**, 1.00 g, 15.1 mmol, 2.00 equiv) in dichloromethane (1.2 mL) at 24 °C. The resulting mixture was stirred for 10 min at 24 °C. Propargyl bromide (80% solution in toluene, 843 μ L, 7.57 mmol, 1 equiv) was added to the stirred solution at 24 °C. The resulting mixture was heated over 10 min to 45 °C, and stirred for 3 h at 45 °C. The product mixture was cooled over 10 min to 24 °C, and diluted sequentially with dichloromethane (50 mL) and water (50 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 \times 50 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 20% ethyl acetate–hexanes, grading to 50% ethyl acetate–hexanes, 3 steps) to afford the deuterated alkyne **S3** as a clear oil (215 mg, 27%).

R_f = 0.06 (20% ethyl acetate–hexanes; CAM). ¹H NMR (400 MHz, CDCl₃): δ 4.20 (d, 2H, *J* = 2.4 Hz, H₁), 2.48 (t, 1H, *J* = 2.4 Hz, H₂), 2.14 (br s, 1H, H₃). ²H NMR (500 MHz, CDCl₃): δ 3.75 (s, 2H, H₅), 3.63 (s, 2H, H₄). ¹³C NMR (500 MHz, CDCl₃): δ 95.0 (CH₂), 79.5 (C), 75.7 (CH), 74.9 (CH₂), 58.5 (CH₂). IR (ATR-FTIR), cm⁻¹: 3279 (br), 1106 (s), 971 (s).



Synthesis of the Silane Probe Mixture 3:

Triethylamine (84.6 μL , 612 μmol , 20.0 equiv) and dichlorodiphenylsilane (32.0 μL , 153 μmol , 5.00 equiv) were added in sequence to a stirred solution of the biotin-CA(PEG)₄-alcohol **S4** (9) (17.2 mg, 30.6 μmol , 1 equiv) in dichloromethane (610 μL) at 24 °C. The resulting solution was stirred for 3 h at 24 °C. The stable isotope mixture (1:3 **S1**:**S2**, 50.3 mg, 498 μmol , 16.2 equiv) was added to the stirred solution at 24 °C. The resulting solution was stirred for an additional 16 h at 24 °C. The product mixture was diluted sequentially with dichloromethane (10 mL) and saturated aqueous sodium bicarbonate solution (10 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 \times 10 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 1% methanol–dichloromethane, grading to 10% methanol–dichloromethane, 3 steps) to afford the silane probe mixture **3** as a clear oil (14.4 mg, 54%).

R_f = 0.39 (5% methanol–dichloromethane; iodine). ^1H NMR (600 MHz, CD_3OD): δ 7.66 (d, 4H, J = 6.6 Hz, H_{18}), 7.42 (t, 2H, J = 5.4 Hz, H_{20}), 7.37 (dd, 4H, J = 7.8 Hz, H_{19}), 4.47 (dd, 1H, J = 7.8, 4.8 Hz, H_2), 4.29 (dd, 1H, J = 7.8, 4.8 Hz, H_5), 4.15 (d, 0.5H, J = 2.4 Hz, H_{23}), 3.92 (t, 2H, J = 4.8 Hz, $\text{H}_{21}/\text{H}_{22}$), 3.72 (t, 2H, J = 5.4 Hz, H_{14}), 3.67 (t, 2H, J = 4.8 Hz, $\text{H}_{21}/\text{H}_{22}$), 3.62–3.52 (m, 16H, $\text{H}_{11}/\text{H}_{12}/\text{H}_{15}$), 3.35 (t, 2H, J = 6.0 Hz, $\text{H}_{11}/\text{H}_{12}$), 3.19 (dt, 1H, J = 9.6, 4.8 Hz, H_6), 2.91 (dd, 1H, J = 12.6, 4.8 Hz, H_1), 2.86–2.84 (m, 1H, H_{24}), 2.70 (d, 1H, J = 12.6 Hz, H_1), 2.48 (t, 2H, J = 6.0 Hz, H_{13}), 2.21 (t, 2H, J = 7.8 Hz, H_{10}), 1.74–1.57 (m, 4H, H_7/H_9), 1.46–1.41 (m, 2H, H_8), 1.25 (s, 6H, $\text{H}_{16}/\text{H}_{17}$). ^{13}C NMR (600 MHz, CD_3OD): δ 176.1 (C), 174.1 (C), 166.1 (C), 136.2 (4 \times CH), 135.7 (2 \times C), 131.3 (2 \times CH), 128.9 (4 \times CH), 77.1 (CH_2), 76.0 (CH_2), 71.8 (CH_2), 71.6 (3 \times CH_2), 71.5 (2 \times CH_2), 71.4 (CH_2), 71.3 (CH_2), 70.6 (CH_2), 68.4 (CH_2), 63.6 (CH), 63.4 (CH_2), 61.6 (CH), 59.0 (C), 57.0 (CH), 51.6 (C), 41.0 (CH_2), 40.4 (CH_2), 37.9 (CH), 36.7 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 28.2 (2 \times CH_3), 26.8 (CH_2). IR (ATR-FTIR), cm^{-1} : 2923 (br), 1645 (m), 1025 (s). HRMS-ESI (m/z): $[\text{M}+\text{Na}]$ calculated for $\text{C}_{42}\text{H}_{62}\text{N}_4\text{O}_{10}\text{SSiNa}$, 865.3854; found, 865.3850. HRMS-ESI (m/z): $[\text{M}+\text{Na}]$ calculated for $\text{C}_{42}\text{H}_{60}\text{D}_2\text{N}_4\text{O}_{10}\text{SSiNa}$, 867.3979; found, 867.3961.

Text of Initial Byonic Glycan Modification Input File:

For Ac₄GalNAz samples:

HexNAz2Si(1) @ OGlycan | common2
HexNAc(1) @ OGlycan | common2
HexNAc(1)HexNAz2Si @ OGlycan | common1
HexNAz2Si(1)Hex(1) @ OGlycan | rare2
HexNAz2Si(1)HexNAc(1)Hex(1) @ OGlycan | rare2
HexNAz2Si(1)Hex(1)NeuAc(1) @ OGlycan | rare2
HexNAz2Si(1)Hex(1)NeuAc(2) @ OGlycan | rare2
HexNAz2Si(1)NeuAc(1) @ OGlycan | rare2
HexNAz2Si(1) @ NGlycan | common1
HexNAz2Si(1)HexNAc(1) @ NGlycan | rare1
HexNAz2Si(1)HexNAc(1)Hex(1) @ NGlycan | rare1
HexNAz2Si(1)HexNAc(1)Hex(3) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(1)Hex(4) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(1)Hex(4) @ NGlycan | common1
HexNAz2Si(1)HexNAc(1)Hex(5) @ NGlycan | common1
HexNAz2Si(1)HexNAc(1)Hex(6) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(1)Hex(7) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(1)Hex(8) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(1)Hex(9) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(2)Hex(3) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(2)Hex(5) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(3)Hex(3) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(3)Hex(4) @ NGlycan | rare2
HexNAz2Si(1)HexNAc(3)Hex(5) @ NGlycan | rare2
HexNAc(1) @ NGlycan | common2
HexNAz(1) @ OGlycan | common1
HexNAz0Si(1) @ OGlycan | common2
HexNAc(1) @ OGlycan | common2
HexNAc(1)HexNAz0Si @ OGlycan | common1
HexNAz0Si(1)Hex(1) @ OGlycan | rare2
HexNAz0Si(1)HexNAc(1)Hex(1) @ OGlycan | rare2
HexNAz0Si(1)Hex(1)NeuAc(1) @ OGlycan | rare2
HexNAz0Si(1)Hex(1)NeuAc(2) @ OGlycan | rare2
HexNAz0Si(1)NeuAc(1) @ OGlycan | rare2
HexNAz0Si(1) @ NGlycan | common1
HexNAz0Si(1)HexNAc(1) @ NGlycan | rare1
HexNAz0Si(1)HexNAc(1)Hex(1) @ NGlycan | rare1
HexNAz0Si(1)HexNAc(1)Hex(3) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(1)Hex(4) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(1)Hex(4) @ NGlycan | common1
HexNAz0Si(1)HexNAc(1)Hex(5) @ NGlycan | common1
HexNAz0Si(1)HexNAc(1)Hex(6) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(1)Hex(7) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(1)Hex(8) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(1)Hex(9) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(2)Hex(3) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(2)Hex(5) @ NGlycan | rare2

HexNAz0Si(1)HexNAc(3)Hex(3) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(3)Hex(4) @ NGlycan | rare2
HexNAz0Si(1)HexNAc(3)Hex(5) @ NGlycan | rare2
% Custom modification text below

For Ac₄ManNAz samples:

HexNAc(1)Hex(1)NeuAz2Si(1) @ OGlycan | common1
HexNAc(1)Hex(1)NeuAz0Si(1) @ OGlycan | common1
HexNAc(1)Hex(1)NeuAz2Si(1)NeuAc(1) @ OGlycan | common1
HexNAc(1)Hex(1)NeuAz2Si(2) @ OGlycan | common1
HexNAc(1)NeuAz2Si(1) @ OGlycan | common1
HexNAc(1)NeuAz0Si(1) @ OGlycan | common1
HexNAc(4)Hex(5)NeuAz2Si(1) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(1)NeuAz2Si(1) @ NGlycan | common1
HexNAc(4)Hex(5)NeuAz2Si(2) @ NGlycan | common1
HexNAc(5)Hex(5)NeuAz2Si(2) @ NGlycan | common1
HexNAc(4)Hex(5)NeuAz2Si(1)NeuAc(1) @ NGlycan | common1
HexNAc(5)Hex(5)NeuAz2Si(1)NeuAc(1) @ NGlycan | common1
HexNAc(4)Hex(5)NeuAz2Si(1)NeuAz(1) @ NGlycan | common1
HexNAc(5)Hex(5)NeuAz2Si(1)NeuAz(1) @ NGlycan | common1

Example output from IsoStamp v2.0.

High Scoring Groups:

Group 1

Group Score 130.3 (best match 112.2, combined match 108.4, bonus 20.0)
Consists of 12 matches with average score 89.7
Average M/Z 692.990 (low 692.988 high 692.991 best 692.991, dev 0.005)
Best Match Range: 3661-3700
Total Range: 3621-3880 (260 scans in range)
Estimated Sulfur: 0.42 (low:0, high:1, best:1, combined: 1)
Tag Type: 102
Mass,Charge: 2078.97, 3
combined match stuff:
scans: 3661-3700
intensity: 69.5 Million
internals1 {linear: 0.129, msqr: 0.120, weighted: 0.169, excess: 0.032, noise: 0.007, missing: 0.000}
internals2 {cor1: 0.844, cor2: 0.855, mz_dev: 0.005, misaligned: 0.258, untagged: 0.304, wrong_tag: 0.021}
scoring:
(BASE SCORE) = 50.0
magnitude 2.0 * +1.84 = +3.7
error(linear) 15.0 * +0.30 = +4.5
error(opt.linear) 5.0 * +0.28 = +1.4
error(wht.linear) 5.0 * +0.20 = +1.0
error(msqr) 15.0 * +0.32 = +4.9
untagged ? 3.0 * +0.27 = +0.8
off by 1 ? 3.0 * +0.37 = +1.1
wrong tag? 1.0 * +0.25 = +0.2
alternate scenario? 8.0 * +0.27 = +2.2
missing spikes 0.0 * +0.15 = +0.0
precursors 4.0 * -0.11 = -0.4
background noise 10.0 * +0.19 = +1.9
m/z deviation 4.0 * +0.56 = +2.3
correlation(2peaks) 10.0 * +0.24 = +2.4
correlation(all peaks) 10.0 * +3.24 = +32.4
mass modifier 0.0 * -0.58 = -0.0
observed: 13 11 230 119 10472 11459 27713 25201 14918 6655 2433 790 167 24 64 0 0 100 0 4
expected: 0 0 0 0 7528 8294 27904 27383 16903 7806 2920 925 256 63 14 3 1 0 0 0
alternate: 0 0 0 0 0 0 24324 27019 16353 7052 2410 691 172 38 8 1 0 0 0 0

Group 2

Group Score 128.0 (best match 104.7, combined match 111.4, bonus 20.0)
Consists of 11 matches with average score 92.9
Average M/Z 1212.207 (low 1212.206 high 1212.210 best 1212.206, dev 0.007)
Best Match Range: 8541-8580
Total Range: 8461-8700 (240 scans in range)
Estimated Sulfur: 2.36 (low:1, high:3, best:3, combined: 3)
Tag Type: 102
Mass,Charge: 3636.62, 3
combined match stuff:
scans: 8541-8580
intensity: 33.5 Million
internals1 {linear: 0.073, msqr: 0.069, weighted: 0.074, excess: 0.020, noise: 0.034, missing: 0.000}
internals2 {cor1: 0.829, cor2: 0.855, mz_dev: 0.007, misaligned: 0.197, untagged: 0.286, wrong_tag: 0.012}
scoring:
(BASE SCORE) = 50.0
magnitude 2.0 * +1.53 = +3.1
error(linear) 15.0 * +0.44 = +6.6
error(opt.linear) 5.0 * +0.39 = +2.0
error(wht.linear) 5.0 * +0.44 = +2.2
error(msqr) 15.0 * +0.45 = +6.8
untagged ? 3.0 * +0.31 = +0.9
off by 1 ? 3.0 * +0.48 = +1.5
wrong tag? 1.0 * +0.25 = +0.2
alternate scenario? 8.0 * +0.31 = +2.5
missing spikes 0.0 * +0.15 = +0.0
precursors 4.0 * -0.16 = -0.6
background noise 10.0 * +0.17 = +1.7

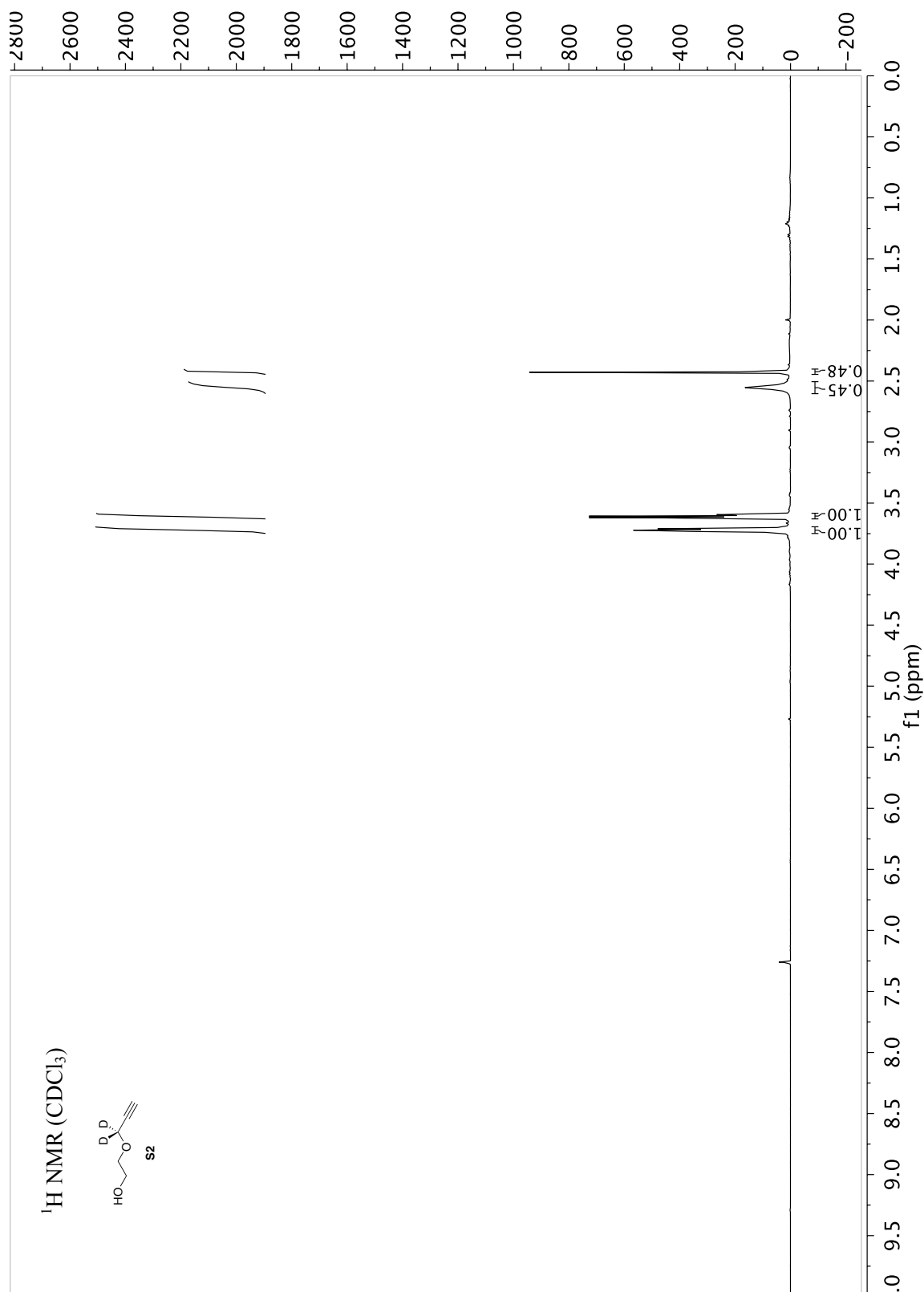
m/z deviation 4.0 * -0.00 = -0.0
 correlation(2peaks) 10.0 * +0.22 = +2.2
 correlation(all peaks) 10.0 * +3.24 = +32.4
 mass modifier 0.0 * -2.14 = -0.0
 observed: 107 27 102 14 1944 7591 15656 21057 20117 15127 9145 5192 2264 897 330 89 77 82 210 221
 expected: 0 0 0 0 2946 5633 14900 21624 21131 15716 9533 4920 2221 895 326 109 34 10 3 1
 alternate: 0 0 0 0 0 11554 22095 23780 18528 11541 6057 2767 1124 412 138 43 12 3 1 0

Low Scoring Group:

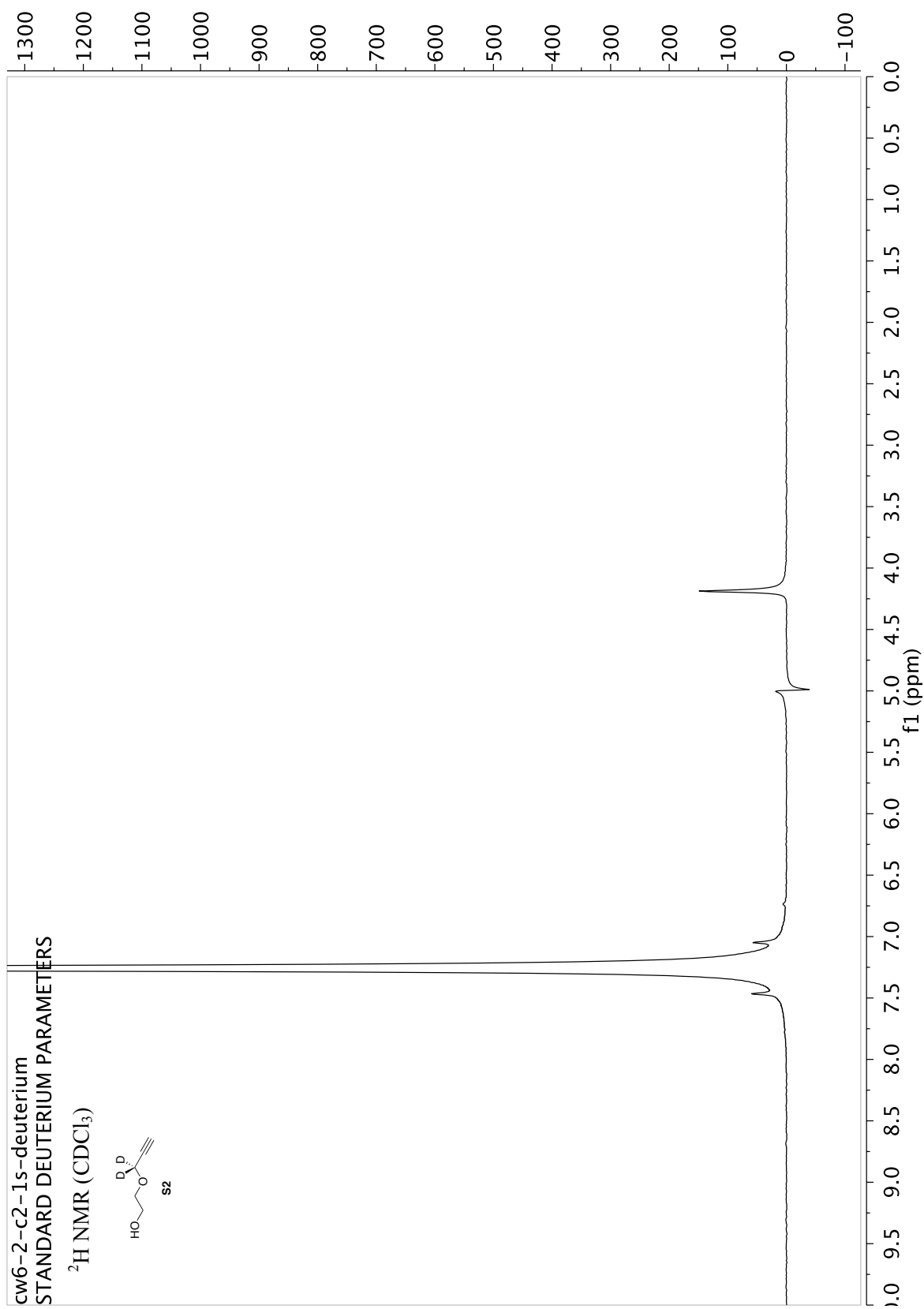
Group 2634

Group Score 10.3 (best match 9.0, combined match 9.9, bonus 0.8)
 Consists of 2 matches with average score -1.8
 Average M/Z 984.692 (low 984.690 high 984.695 best 984.695, dev 0.019)
 Best Match Range: 8561-8600
 Total Range: 8561-8620 (60 scans in range)
 Estimated Sulfur: 3.00 (low:3, high:3, best:3, combined: 3)
 Tag Type: 102
 Mass,Charge: 3938.77, 4
 combined match stuff:
 scans: 8561-8600
 intensity: 1.2 Million
 internals1 {linear: 0.362, msqr: 0.282, weighted: 0.269, excess: 0.039, noise: 0.362, missing: 0.012}
 internals2 {cor1: 0.364, cor2: 0.827, mz_dev: 0.019, misaligned: 0.693, untagged: 0.834, wrong_tag: 0.050}
 scoring:
 (BASE SCORE) = 50.0
 magnitude 2.0 * +0.10 = +0.2
 error(linear) 15.0 * -0.28 = -4.2
 error(opt.linear) 5.0 * -0.28 = -1.4
 error(wht.linear) 5.0 * -0.05 = -0.2
 error(msqr) 15.0 * -0.08 = -1.2
 untagged ? 3.0 * -2.14 = -6.4
 off by 1 ? 3.0 * -1.28 = -3.8
 wrong tag? 1.0 * +0.24 = +0.2
 alternate scenario? 8.0 * -2.14 = -17.2
 missing spikes 0.0 * +0.09 = +0.0
 precursors 4.0 * -3.64 = -14.6
 background noise 10.0 * -0.16 = -1.6
 m/z deviation 4.0 * -4.07 = -16.3
 correlation(2peaks) 10.0 * -0.53 = -5.3
 correlation(all peaks) 10.0 * +3.17 = +31.7
 mass modifier 0.0 * -2.44 = -0.0
 observed: 1006 454 3412 0 2287 4221 14085 17644 16053 10694 10140 8147 3348 0 2039 4022 2477 1208 2375 1260
 expected: 0 0 0 0 2484 5154 13389 20381 21057 16560 10609 5775 2748 1166 448 157 51 15 4 1
 alternate: 0 0 0 0 0 9709 20144 23204 19229 12690 7040 3393 1453 562 198 65 20 6 1 0

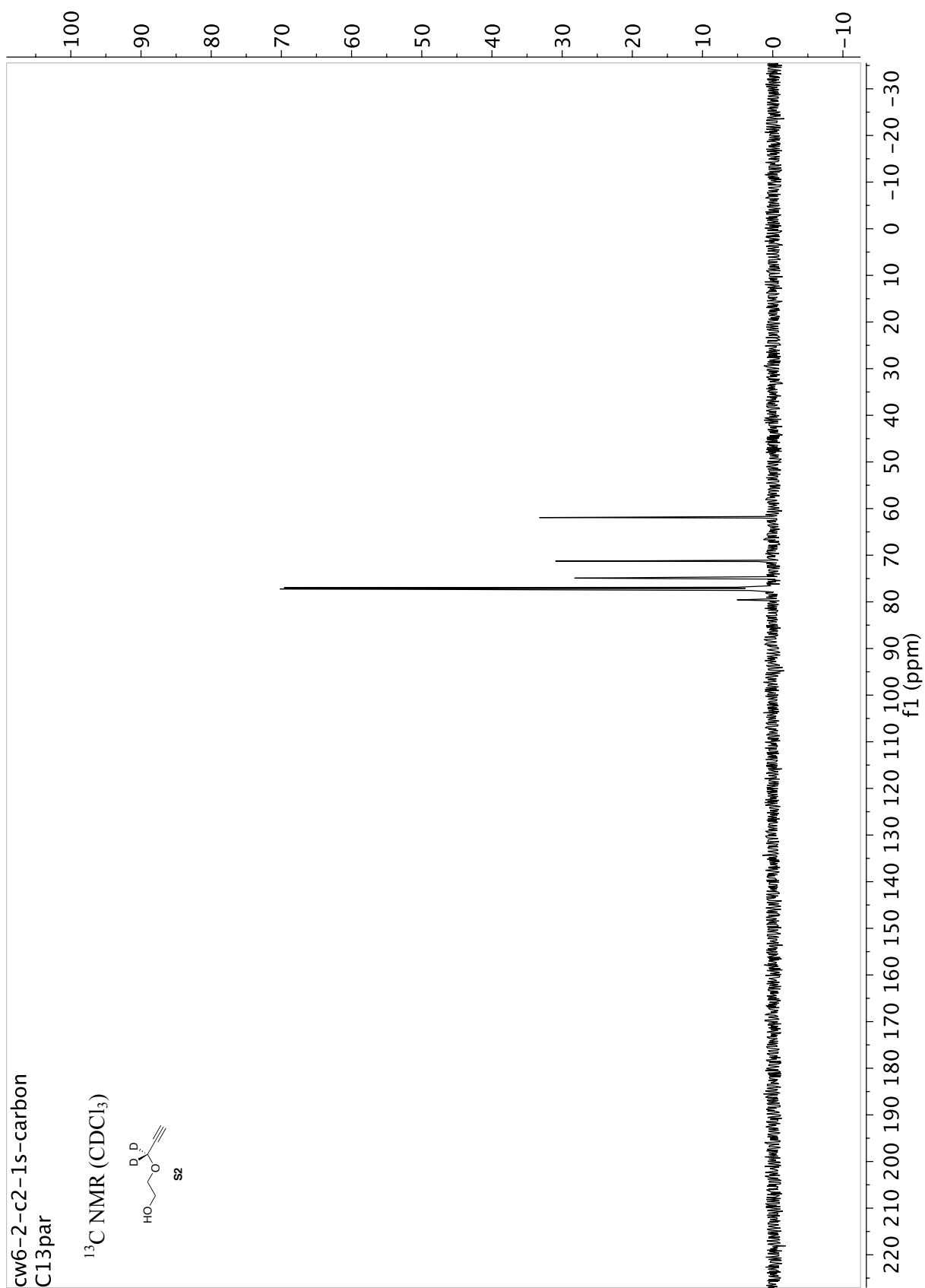
Catalog of Nuclear Magnetic Resonance and Infrared Spectra.



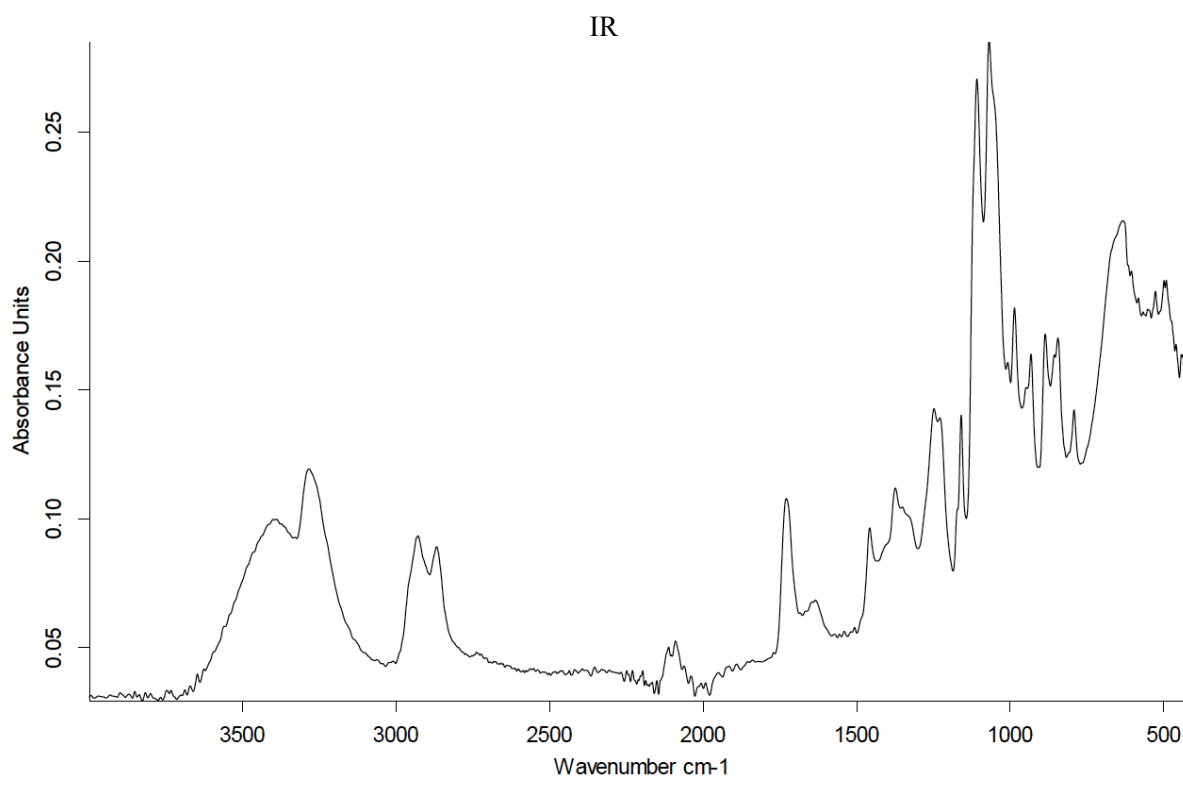
Woo, C. M., et al. "Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes." *J. Prot. Res.* 2017.

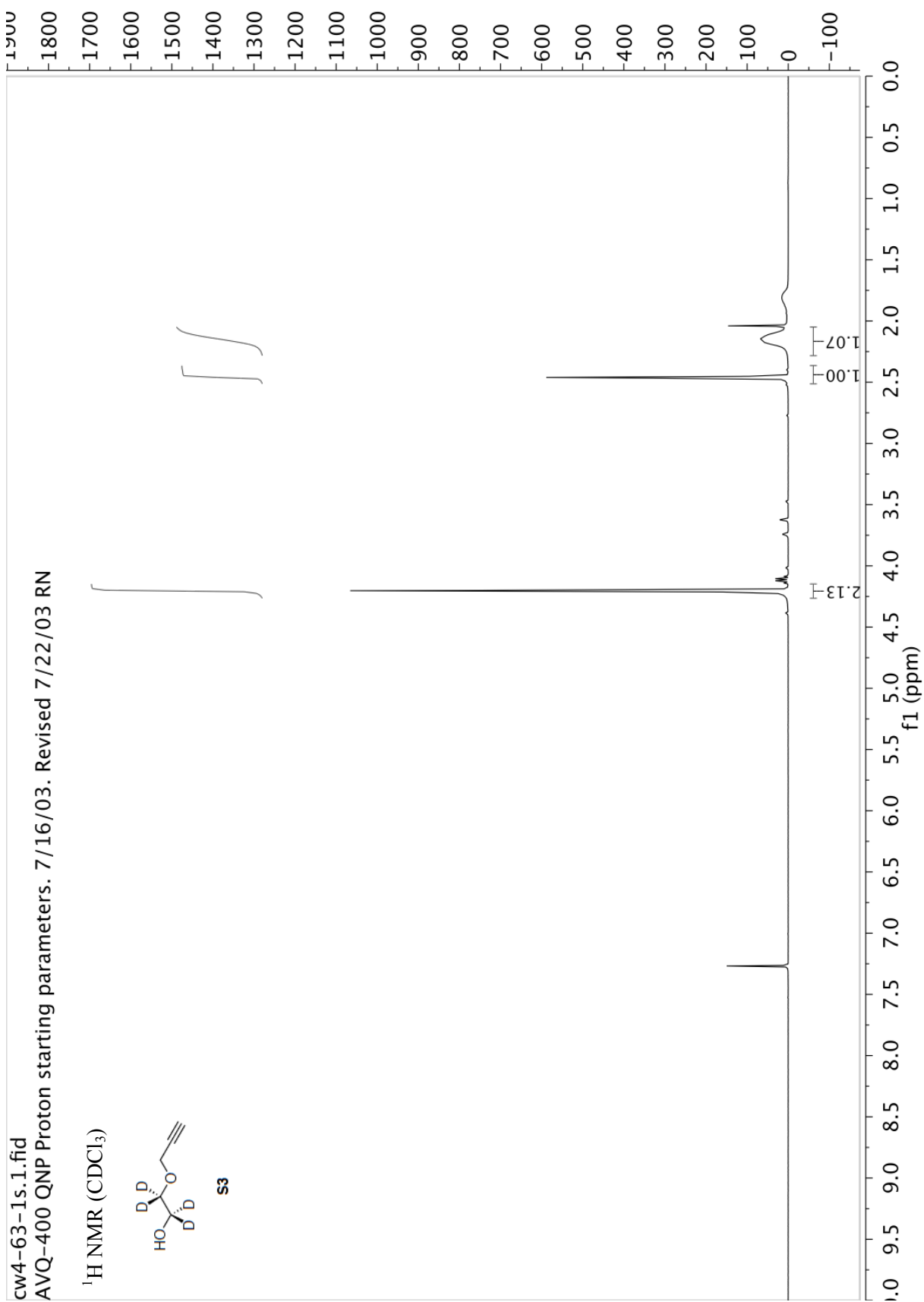


Woo, C. M., et al. "Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes." *J. Prot. Res.* 2017.

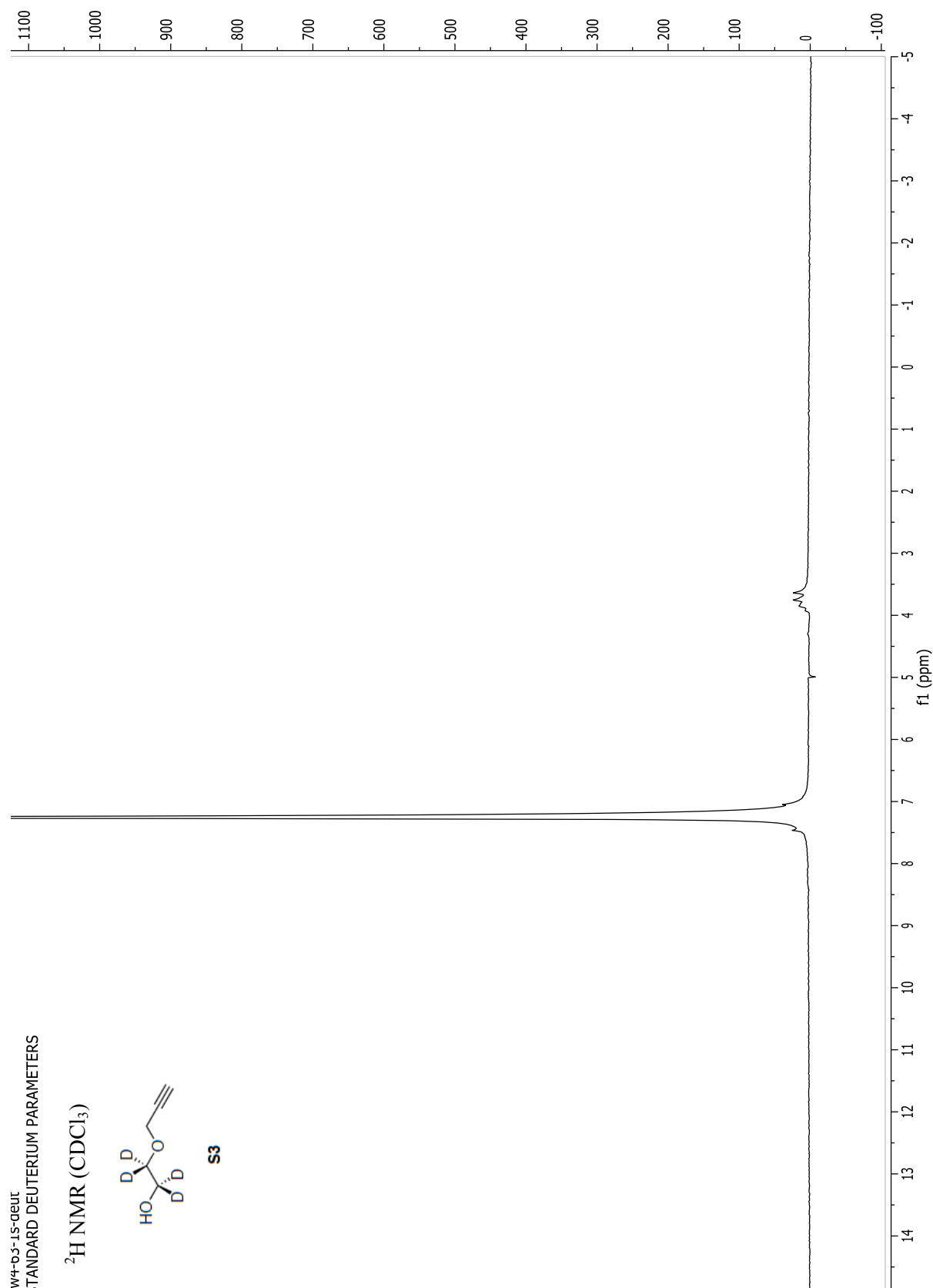


Woo, C. M., et al. "Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes." *J. Prot. Res.* 2017.

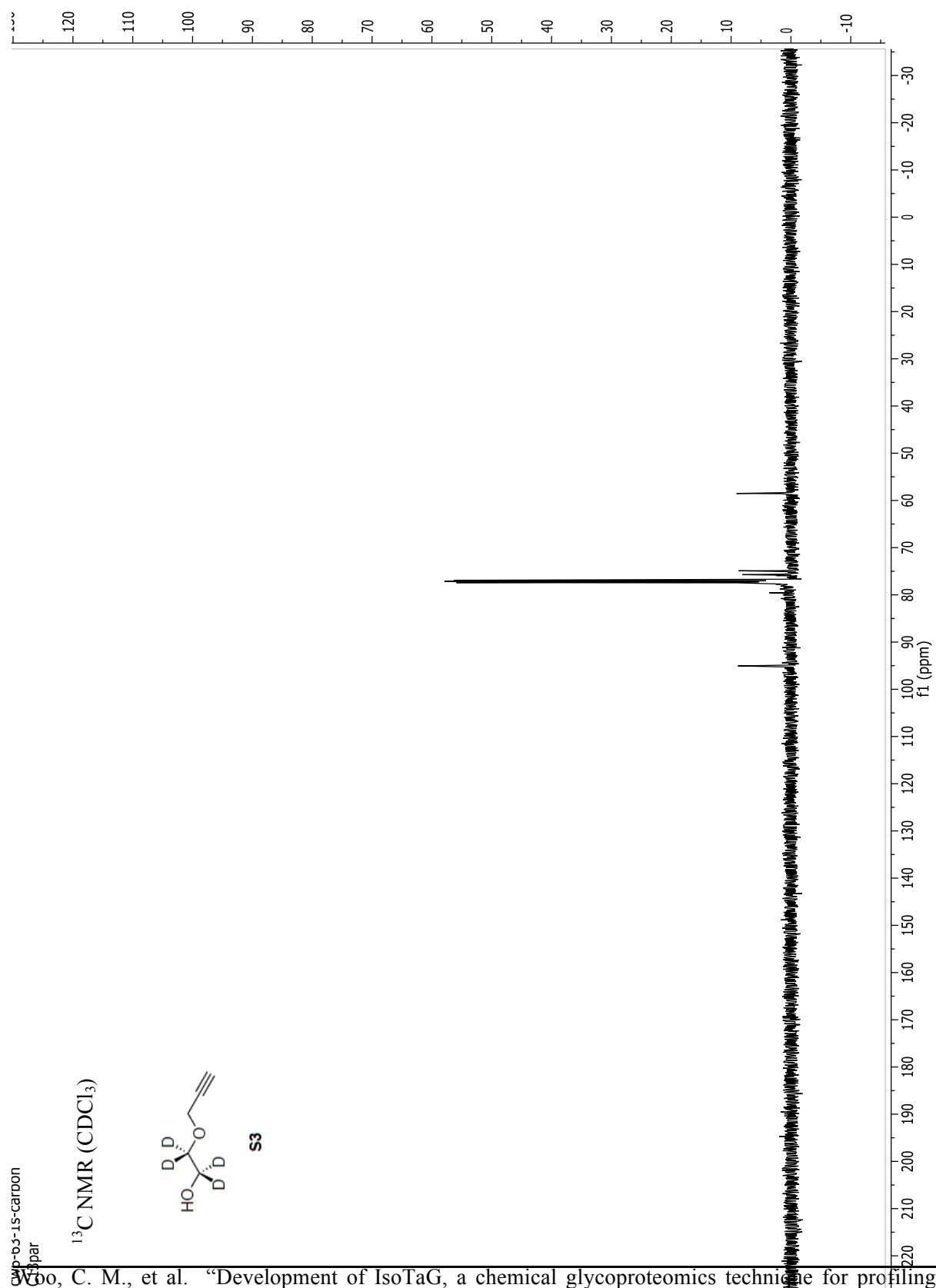




Woo, C. M., et al. "Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes." *J. Prot. Res.* 2017.

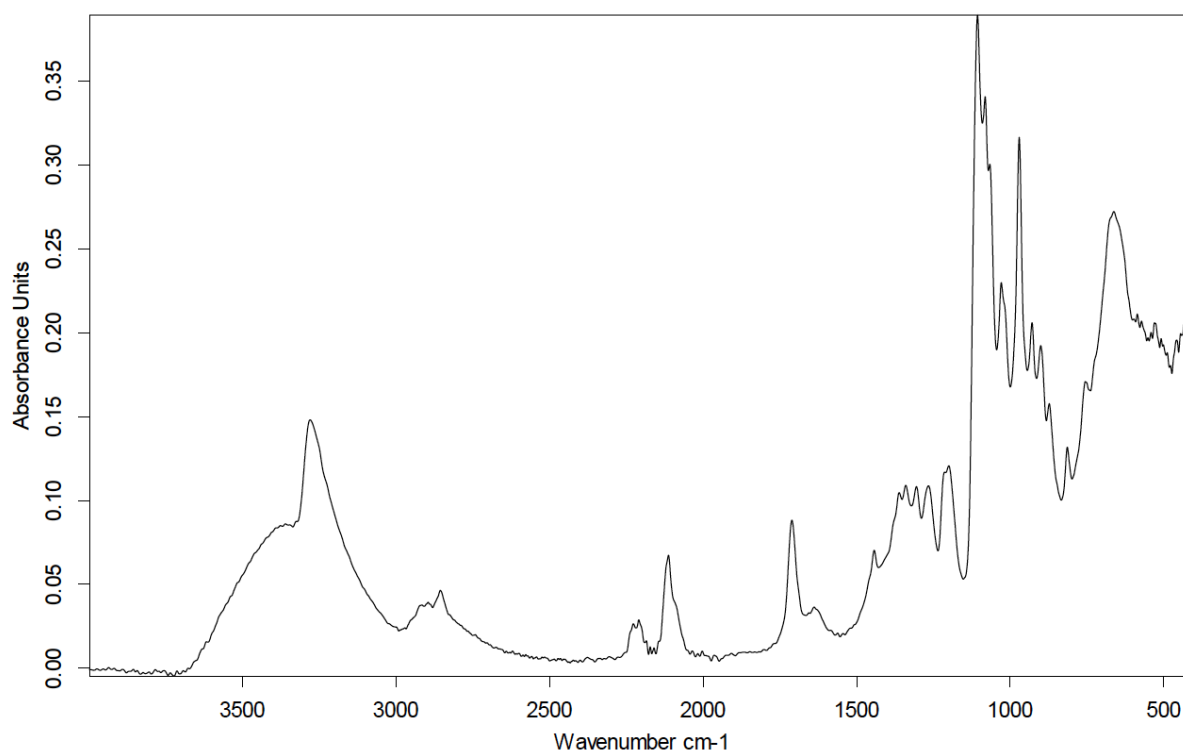


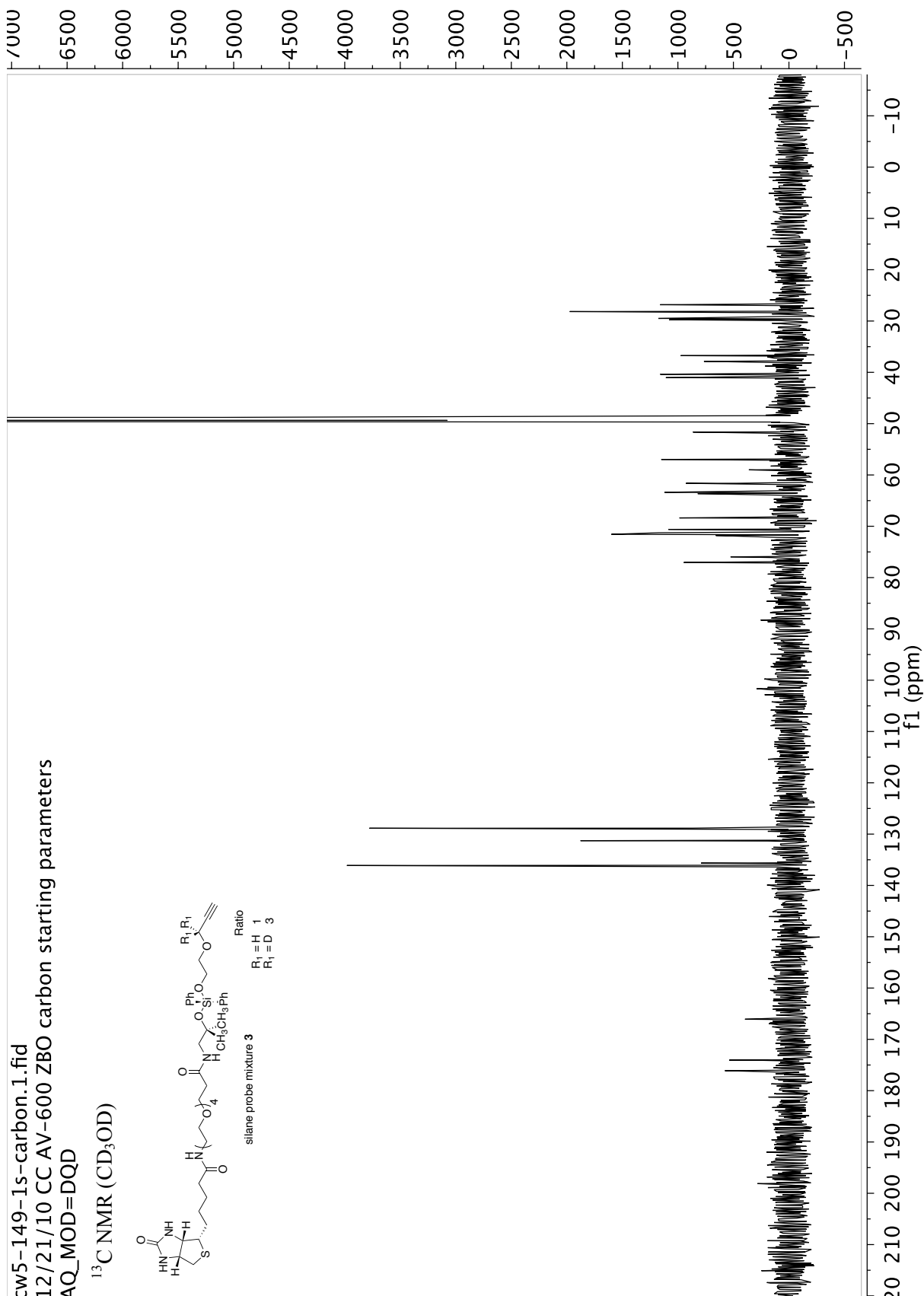
Woo, C. M., et al. "Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes." *J. Prot. Res.* 2017.



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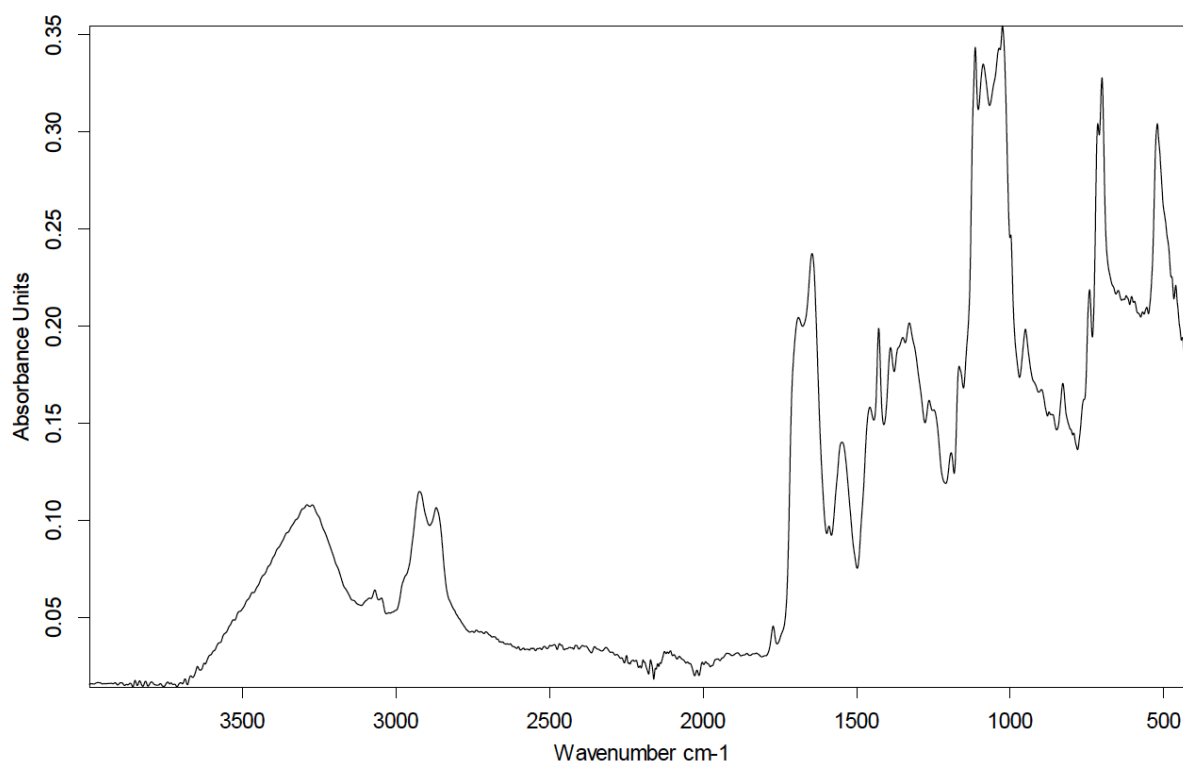
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Woo, C. M., et al. "Development of IsoTaG, a chemical glycoproteomics technique for profiling intact N- and O-glycopeptides from whole cell proteomes." *J. Prot. Res.* 2017.

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Bibliography.

1. Palaniappan, K. K., Pitcher, A. A., Smart, B. P., Spiciarich, D. R., Iavarone, A. T., and Bertozzi, C. R. (2011) *ACS Chem. Biol.* **6**, 829-836.
2. Woo, C. M., Iavarone, A. T., Spiciarich, D. R., Palaniappan, K. K., and Bertozzi, C. R. (2015) *Nat Meth* **12**, 561-567.
3. Still, W. C., Kahn, M., and Mitra, A. (1978). *J. Org. Chem.* **43**, 2923.
4. Pangborn, A. B., Giardello, M. A., Grubbs, R. H., Rosen, R. K., and Timmers, F. J. (1996). *Organometallics* **15**, 1518.
5. Lee, P. J. J., and Compton, B. J. (2007) Patent, U. S. ed., Waters Investments Limited, USA.
6. Wang, W., Hong, S., Tran, A., Jiang, H., Triano, R., Liu, Y., Chen, X., and Wu, P. (2011) *Chem Asian J* **6**, 2796-2802.
7. Hang, H. C., Yu, C., Kato, D. L., and Bertozzi, C. R. (2003) *Proc Natl Acad Sci* **100**, 14846-14851.
8. Prescher, J. A., Dube, D. H., and Bertozzi, C. R. (2004) *Nature* **430**, 873-877.
9. Szychowski, J., Mahdavi, A., Hodas, J. J., Bagert, J. D., Ngo, J. T., Landgraf, P., Dieterich, D. C., Schuman, E. M., and Tirrell, D. A. (2010) *J Am Chem Soc* **132**, 18351-18360.